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Investigating the etiology of bovine digital dermatitis by a combination of 16S rRNA gene analysis and fluorescence *in situ* hybridization

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Bovine digital dermatitis, the cause of lameness and wasting in cattle, was first reported in 1974. Today, this disease has considerable negative effects on animal welfare and production economy in many parts of the world. A bacterial etiology of digital dermatitis is now well documented, and the current view on this disease points towards a complicated etiology involving co-infection of more than one, and probably multiple species belonging to the genus *Treponema*.
Still, the pathogenic role of each of the digital dermatitis-associated phylotypes remains unclear. The aim of this investigation was to obtain a better understanding of digital dermatitis in general, including possible predisposing skin alternations and the role of the bacteria *Dichelobacter nodosus*. Finally, we wanted to determine if any *Treponema* phylotypes could be singled out as having a particularly prominent role in the etiology of the disease.
Here, a PCR-based approach targeting the 16S rRNA gene along with fluorescence *in situ* hybridization was used to determine the prevalence and diversity of 17 *Treponema* phylotypes in 85 digital dermatitis lesions from six Danish dairy herds as well as additional biopsies of healthy skin and previously examined digital dermatitis lesions. All skin samples were evaluated histopathologically for possible predisposing abnormalities. Furthermore, fluorescence *in situ* hybridization tests for *Fusobacterium. necrophorum* and *D. nodosus* was applied.
All lesions revealed intermingled infections with multiple *Treponema* phylotypes (mean > 7). In six herds, the mean number of phylotypes identified varied between 12 and 15. *D. nodosus* was present in forty-nine (51%) of the lesions and in three of the apparently healthy skin samples. One "healthy" sample also contained *Treponema* spp. and *D. nodosus*, and were histopathologically categorized as subclinical digital dermatitis.
We propose that external noxious stimuli allow *D. nodosus* to break down the epidermal barrier creating a suitable environment for the secondary invaders, *Treponema* species, which gradually take over the infection site. The variety and different distribution of treponemes in the digital dermatitis lesions observed in this study, suggests that most of the *Treponema* phylotypes have the potential to be pathogenic.